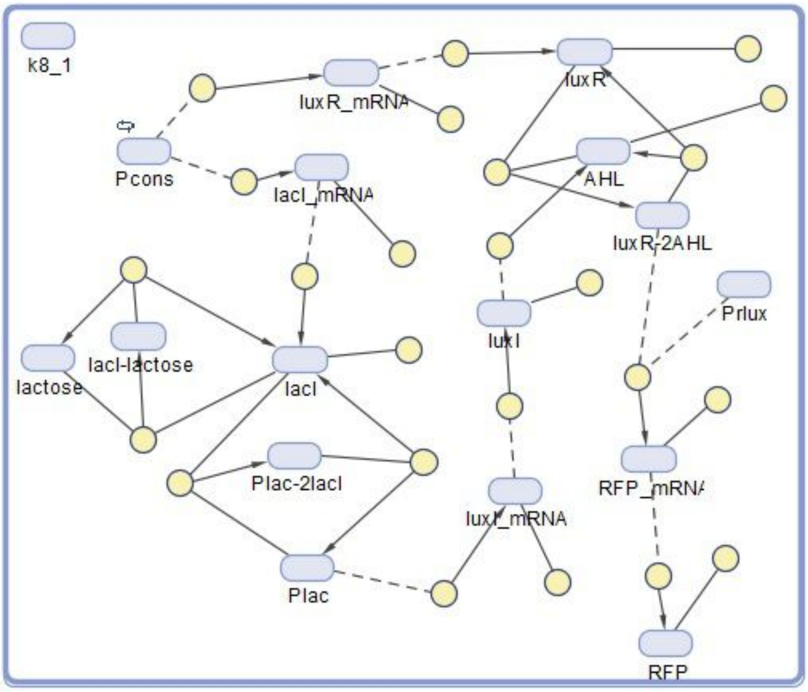
Modeling Supplement

1. The positive feedback system[1]

(1) .control:



a. ODEs[2] [3]:

d(Ecoli_1.lacI)/dt = 1/Ecoli_1*(ReactionFlux2 - ReactionFlux3 + ReactionFlux4 - -2*ReactionFlux5 + 2*ReactionFlux6 - ReactionFlux8)

d(Ecoli_1.luxI)/dt = 1/Ecoli_1*(-ReactionFlux11 + ReactionFlux15)

d(Ecoli_1.luxR)/dt = 1/Ecoli_1*(-ReactionFlux14 + ReactionFlux18 - ReactionFlux19 + ReactionFlux22)

d(Ecoli_1.AHL)/dt = 1/Ecoli_1*(-ReactionFlux12 + ReactionFlux16 - -2*ReactionFlux19 + 2*ReactionFlux22)

d(Ecoli_1.RFP)/dt = 1/Ecoli_1*(ReactionFlux21 - ReactionFlux23)

d(Ecoli_1.lacI_mRNA)/dt = 1/Ecoli_1*(ReactionFlux1 - ReactionFlux9)

d(Ecoli_1.luxI_mRNA)/dt = 1/Ecoli_1*(ReactionFlux7 - ReactionFlux10)

d(Ecoli_1.luxR_mRNA)/dt = 1/Ecoli_1*(-ReactionFlux13 + ReactionFlux17)

d(Ecoli_1.RFP_mRNA)/dt = 1/Ecoli_1*(ReactionFlux20 - ReactionFlux97)

d(Ecoli_1.lactose)/dt = 1/Ecoli_1*(-ReactionFlux3 + ReactionFlux4)

d(Ecoli_1.[lacI-lactose])/dt = 1/Ecoli_1*(ReactionFlux3 - ReactionFlux4)

d(Ecoli_1.Plac)/dt = 1/Ecoli_1*(-ReactionFlux5 + ReactionFlux6)

d(Ecoli_1.[Plac-2lacI])/dt = 1/Ecoli_1*(ReactionFlux5 - ReactionFlux6)

d(Ecoli_1.[luxR-2AHL])/dt = 1/Ecoli_1*(ReactionFlux19 - ReactionFlux22)

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[1] Information about modeling and more results.

[2] The species in the equations always stand for the concentration of the substances.

[3] All Simbiology files can be found in the attachments.

b. Fluxes:

ReactionFlux1 = (k1*N)*Ecoli_1

ReactionFlux2 = (Kt*Ecoli_1.lacI_mRNA)*Ecoli_1

ReactionFlux3 = (K1on*Ecoli_1.lacI*Ecoli_1.lactose)*Ecoli_1

ReactionFlux4 = (K1off*Ecoli_1.[lacI-lactose])*Ecoli_1

ReactionFlux5 = (K3on*Ecoli_1.lacI*Ecoli_1.lacI*Ecoli_1.Plac)*Ecoli_1

ReactionFlux6 = (K3off*Ecoli_1.[Plac-2lacI])*Ecoli_1

ReactionFlux7 = (k7*(Ecoli_1.Plac/(Ecoli_1.Plac+Ecoli_1.[Plac-2lacI]))*N)*Ecoli_1

ReactionFlux8 = (beta_1*Ecoli_1.lacI)*Ecoli_1

ReactionFlux9 = (beta_3*Ecoli_1.lacI_mRNA)*Ecoli_1

ReactionFlux10 = (beta_3*Ecoli_1.luxI_mRNA)*Ecoli_1

ReactionFlux11 = (beta_6*Ecoli_1.luxI)*Ecoli_1

ReactionFlux12 = (beta_2*Ecoli_1.AHL)*Ecoli_1

ReactionFlux13 = (beta_3*Ecoli_1.luxR_mRNA)*Ecoli_1

ReactionFlux14 = (beta_7*Ecoli_1.luxR)*Ecoli_1

ReactionFlux15 = (Kt*Ecoli_1.luxI_mRNA)*Ecoli_1

ReactionFlux16 = (k15*Ecoli_1.luxI)*Ecoli_1

ReactionFlux17 = (k1*N)*Ecoli_1

ReactionFlux18 = (Kt*Ecoli_1.luxR_mRNA)*Ecoli_1

ReactionFlux19 = (K5on*Ecoli_1.luxR*Ecoli_1.AHL*Ecoli_1.AHL)*Ecoli_1

ReactionFlux20 = (k8_1*N*kp)*Ecoli_1

ReactionFlux21 = (Kt*Ecoli_1.RFP_mRNA)*Ecoli_1

ReactionFlux22 = (K5off*Ecoli_1.[luxR-2AHL])*Ecoli_1

ReactionFlux23 = (beta_8*Ecoli_1.RFP)*Ecoli_1

ReactionFlux97 = (beta_3*Ecoli_1.RFP_mRNA)*Ecoli_1

c. Initial Conditions[4]:

Ecoli_1.lacI = 6e-05 molarity

Ecoli_1.luxI = 0 molarity

Ecoli_1.luxR = 6e-05 molarity

Ecoli_1.AHL = 0 molarity

Ecoli_1.RFP = 0 molarity

Ecoli_1.lacI_mRNA = 1.6e-05 molarity

Ecoli_1.luxI_mRNA = 0 molarity

Ecoli_1.luxR_mRNA = 1.6e-05 molarity

Ecoli_1.RFP_mRNA = 0 molarity

Ecoli_1.lactose = 0.0023 molarity

Ecoli_1.[lacI-lactose] = 0 molarity

Ecoli_1.Plac = 0 molarity

Ecoli_1.[Plac-2lacI] = 4e-09 molarity

Ecoli_1.[luxR-2AHL] = 0 molarity

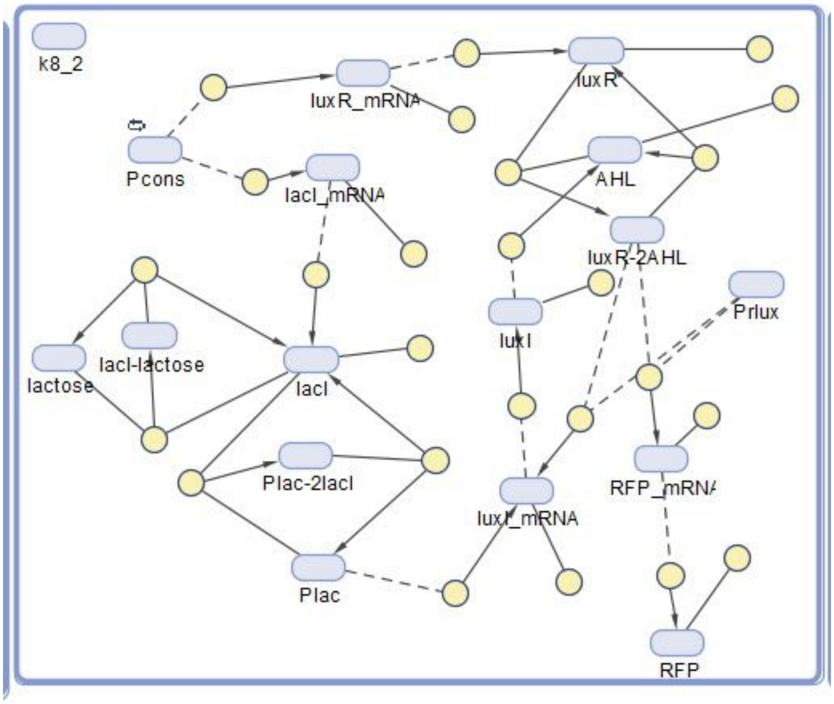
Ecoli_1.Prlux = 4e-09 molarity

k8_1 = 5e-06 molarity

[4] Initial values here stand for the concentrations of the substances, before adding lactose.

d. when Ecoli_1.[luxR-2AHL] > Km, set k8_1 = k8

(2).luxI+



a. ODEs:

d(Ecoli_2.lacI)/dt = 1/Ecoli_2*(-ReactionFlux39 + 2*ReactionFlux41 - -2*ReactionFlux42 + ReactionFlux43 - ReactionFlux44 + ReactionFlux45)

d(Ecoli_2.luxI)/dt = 1/Ecoli_2*(ReactionFlux32 - ReactionFlux36)

d(Ecoli_2.luxR)/dt = 1/Ecoli_2*(ReactionFlux25 - ReactionFlux28 + ReactionFlux29 - ReactionFlux33)

d(Ecoli_2.AHL)/dt = 1/Ecoli_2*(2*ReactionFlux25 - -2*ReactionFlux28 + ReactionFlux31 - ReactionFlux35)

d(Ecoli_2.RFP)/dt = 1/Ecoli_2*(-ReactionFlux24 + ReactionFlux26)

d(Ecoli_2.lacI_mRNA)/dt = 1/Ecoli_2*(-ReactionFlux38 + ReactionFlux46)

d(Ecoli_2.luxI_mRNA)/dt = 1/Ecoli_2*(-ReactionFlux37 + ReactionFlux40 + ReactionFlux93)

d(Ecoli_2.luxR_mRNA)/dt = 1/Ecoli_2*(ReactionFlux30 - ReactionFlux34)

d(Ecoli_2.RFP_mRNA)/dt = 1/Ecoli_2*(ReactionFlux27 - ReactionFlux98)

d(Ecoli_2.lactose)/dt = 1/Ecoli_2*(ReactionFlux43 - ReactionFlux44)

d(Ecoli_2.[lacI-lactose])/dt = 1/Ecoli_2*(-ReactionFlux43 + ReactionFlux44)

d(Ecoli_2.Plac)/dt = 1/Ecoli_2*(ReactionFlux41 - ReactionFlux42)

d(Ecoli_2.[Plac-2lacI])/dt = 1/Ecoli_2*(-ReactionFlux41 + ReactionFlux42)

d(Ecoli_2.[luxR-2AHL])/dt = 1/Ecoli_2*(-ReactionFlux25 + ReactionFlux28)

d(Ecoli_3.lacI)/dt = 1/Ecoli_3*(-ReactionFlux62 + 2*ReactionFlux64 - -2*ReactionFlux65 + ReactionFlux66 - ReactionFlux67 + ReactionFlux68)

b. Fluxes:

ReactionFlux24 = (beta_8*Ecoli_2.RFP)*Ecoli_2

ReactionFlux25 = (K5off*Ecoli_2.[luxR-2AHL])*Ecoli_2

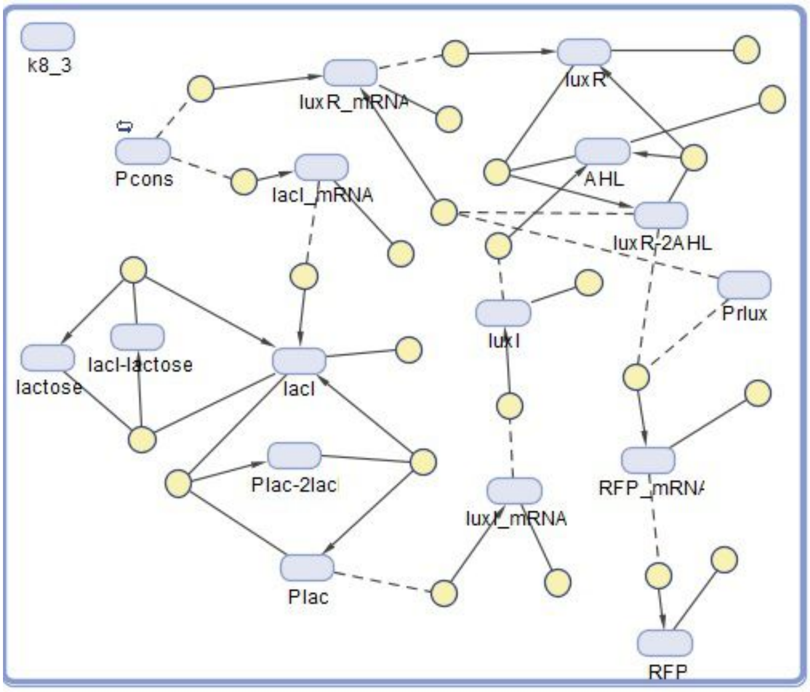
ReactionFlux26 = (Kt*Ecoli_2.RFP_mRNA)*Ecoli_2
ReactionFlux27 = (k8_2*N*kp)*Ecoli_2
ReactionFlux28 = (K5on*Ecoli_2.luxR*Ecoli_2.AHL*Ecoli_2.AHL)*Ecoli_2
ReactionFlux29 = (Kt*Ecoli_2.luxR_mRNA)*Ecoli_2
ReactionFlux30 = (k1*N)*Ecoli_2
ReactionFlux31 = (k15*Ecoli_2.luxI)*Ecoli_2
ReactionFlux32 = (Kt*Ecoli_2.luxI_mRNA)*Ecoli_2
ReactionFlux33 = (beta_7*Ecoli_2.luxR)*Ecoli_2
ReactionFlux34 = (beta_3*Ecoli_2.luxR_mRNA)*Ecoli_2
ReactionFlux35 = (beta_2*Ecoli_2.AHL)*Ecoli_2
ReactionFlux36 = (beta_6*Ecoli_2.luxI)*Ecoli_2
ReactionFlux37 = (beta_3*Ecoli_2.luxI_mRNA)*Ecoli_2
ReactionFlux38 = (beta_3*Ecoli_2.lacI_mRNA)*Ecoli_2
ReactionFlux39 = (beta_1*Ecoli_2.lacI)*Ecoli_2
ReactionFlux40 = (k7*(Ecoli_2.Plac/(Ecoli_2.Plac+Ecoli_2.[Plac-2lacI]))*N)*Ecoli_2
ReactionFlux41 = (K3off*Ecoli_2.[Plac-2lacI])*Ecoli_2
ReactionFlux42 = (K3on*Ecoli_2.lacI*Ecoli_2.lacI*Ecoli_2.Plac)*Ecoli_2
ReactionFlux43 = (K1off*Ecoli_2.[lacI-lactose])*Ecoli_2
ReactionFlux44 = (K1on*Ecoli_2.lacI*Ecoli_2.lactose)*Ecoli_2
ReactionFlux45 = (Kt*Ecoli_2.lacI_mRNA)*Ecoli_2
ReactionFlux46 = (k1*N)*Ecoli_2
ReactionFlux98 = (beta_3*Ecoli_2.RFP_mRNA)*Ecoli_2
ReactionFlux93 = (k8_2*N*kp)*Ecoli_2

c. Initial conditions:

Ecoli_2.lacI = 6e-05 molarity
Ecoli_2.luxI = 0 molarity
Ecoli_2.luxR = 6e-05 molarity
Ecoli_2.AHL = 0 molarity
Ecoli_2.RFP = 0 molarity
Ecoli_2.lacI_mRNA = 1.6e-05 molarity
Ecoli_2.luxI_mRNA = 0 molarity
Ecoli_2.luxR_mRNA = 1.6e-05 molarity
Ecoli_2.RFP_mRNA = 0 molarity
Ecoli_2.lactose = 0.0023 molarity
Ecoli_2.[lacI-lactose] = 0 molarity
Ecoli_2.Plac = 0 molarity
Ecoli_2.[Plac-2lacI] = 4e-09 molarity
Ecoli_2.[luxR-2AHL] = 0 molarity
Ecoli_2.Prlux = 4e-09 molarity
k8_2 = 5e-06 molarity

d. when Ecoli_2.[luxR-2AHL] > Km, set k8_2 = k8

(3).luxR+



a. ODEs:

d(Ecoli_3.luxI)/dt = 1/Ecoli_3*(ReactionFlux55 - ReactionFlux59)

d(Ecoli_3.luxR)/dt = 1/Ecoli_3*(ReactionFlux48 - ReactionFlux51 + ReactionFlux52 - ReactionFlux56)

d(Ecoli_3.AHL)/dt = 1/Ecoli_3*(2*ReactionFlux48 - -2*ReactionFlux51 + ReactionFlux54 - ReactionFlux58)

d(Ecoli_3.RFP)/dt = 1/Ecoli_3*(-ReactionFlux47 + ReactionFlux49)

d(Ecoli_3.lacI_mRNA)/dt = 1/Ecoli_3*(-ReactionFlux61 + ReactionFlux69)

d(Ecoli_3.luxI_mRNA)/dt = 1/Ecoli_3*(-ReactionFlux60 + ReactionFlux63)

d(Ecoli_3.luxR_mRNA)/dt = 1/Ecoli_3*(ReactionFlux53 - ReactionFlux57 + ReactionFlux94)

d(Ecoli_3.RFP_mRNA)/dt = 1/Ecoli_3*(ReactionFlux50 - ReactionFlux99)

d(Ecoli_3.lactose)/dt = 1/Ecoli_3*(ReactionFlux66 - ReactionFlux67)

d(Ecoli_3.[lacI-lactose])/dt = 1/Ecoli_3*(-ReactionFlux66 + ReactionFlux67)

d(Ecoli_3.Plac)/dt = 1/Ecoli_3*(ReactionFlux64 - ReactionFlux65)

d(Ecoli_3.[Plac-2lacI])/dt = 1/Ecoli_3*(-ReactionFlux64 + ReactionFlux65)

d(Ecoli_3.[luxR-2AHL])/dt = 1/Ecoli_3*(-ReactionFlux48 + ReactionFlux51)

b. Fluxes:

ReactionFlux47 = (beta_8*Ecoli_3.RFP)*Ecoli_3

ReactionFlux48 = (K5off*Ecoli_3.[luxR-2AHL])*Ecoli_3

ReactionFlux49 = (Kt*Ecoli_3.RFP_mRNA)*Ecoli_3

ReactionFlux50 = (k8_3*N*kp)*Ecoli_3

ReactionFlux51 = (K5on*Ecoli_3.luxR*Ecoli_3.AHL*Ecoli_3.AHL)*Ecoli_3

ReactionFlux52 = (Kt*Ecoli_3.luxR_mRNA)*Ecoli_3

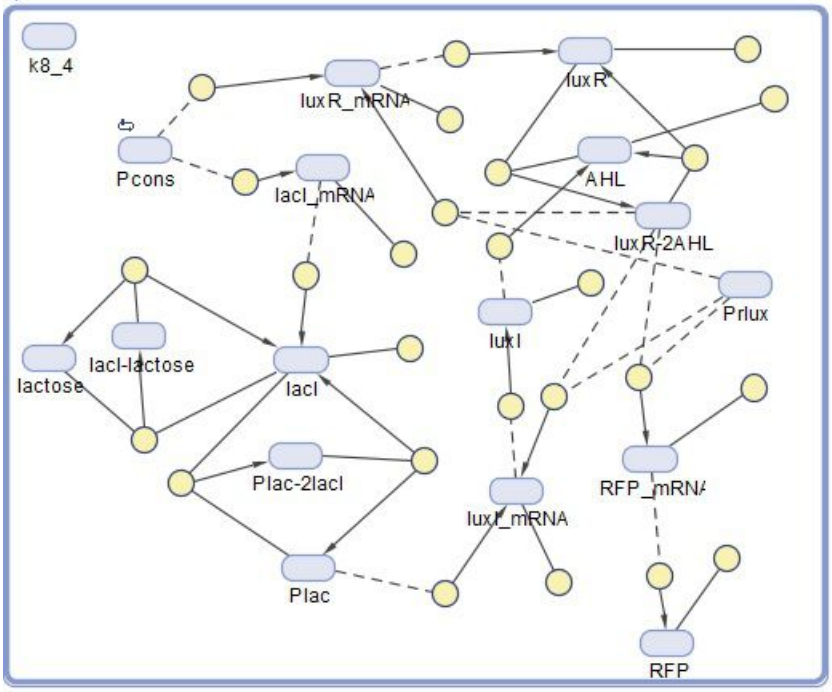
ReactionFlux53 = (k1*N)*Ecoli_3
ReactionFlux54 = (k15*Ecoli_3.luxI)*Ecoli_3
ReactionFlux55 = (Kt*Ecoli_3.luxI_mRNA)*Ecoli_3
ReactionFlux56 = (beta_7*Ecoli_3.luxR)*Ecoli_3
ReactionFlux57 = (beta_3*Ecoli_3.luxR_mRNA)*Ecoli_3
ReactionFlux58 = (beta_2*Ecoli_3.AHL)*Ecoli_3
ReactionFlux59 = (beta_6*Ecoli_3.luxI)*Ecoli_3
ReactionFlux60 = (beta_3*Ecoli_3.luxI_mRNA)*Ecoli_3
ReactionFlux61 = (beta_3*Ecoli_3.lacI_mRNA)*Ecoli_3
ReactionFlux62 = (beta_1*Ecoli_3.lacI)*Ecoli_3
ReactionFlux63 = (k7*(Ecoli_3.Plac/(Ecoli_3.Plac+Ecoli_3.[Plac-2lacI]))*N)*Ecoli_3
ReactionFlux64 = (K3off*Ecoli_3.[Plac-2lacI])*Ecoli_3
ReactionFlux65 = (K3on*Ecoli_3.lacI*Ecoli_3.lacI*Ecoli_3.Plac)*Ecoli_3
ReactionFlux66 = (K1off*Ecoli_3.[lacI-lactose])*Ecoli_3
ReactionFlux67 = (K1on*Ecoli_3.lacI*Ecoli_3.lactose)*Ecoli_3
ReactionFlux68 = (Kt*Ecoli_3.lacI_mRNA)*Ecoli_3
ReactionFlux69 = (k1*N)*Ecoli_3
ReactionFlux94 = (k8_3*kp*N)*Ecoli_3
ReactionFlux99 = (beta_3*Ecoli_3.RFP_mRNA)*Ecoli_3

c. Initial conditions:

Ecoli_3.lacI = 6e-05 molarity
Ecoli_3.luxI = 0 molarity
Ecoli_3.luxR = 6e-05 molarity
Ecoli_3.AHL = 0 molarity
Ecoli_3.RFP = 0 molarity
Ecoli_3.lacI_mRNA = 1.6e-05 molarity
Ecoli_3.luxI_mRNA = 0 molarity
Ecoli_3.luxR_mRNA = 1.6e-05 molarity
Ecoli_3.RFP_mRNA = 0 molarity
Ecoli_3.lactose = 0.0023 molarity
Ecoli_3.[lacI-lactose] = 0 molarity
Ecoli_3.Plac = 0 molarity
Ecoli_3.[Plac-2lacI] = 4e-09 molarity
Ecoli_3.[luxR-2AHL] = 0 molarity
Ecoli_3.Prlux = 4e-09 molarity
k8_3 = 5e-06 molarity

d. when Ecoli_3.[luxR-2AHL] > Km, set k8_3 = k8

(4). lux+ and luxR+



a. ODEs:

d(Ecoli_4.lacI)/dt = 1/Ecoli_4*(-ReactionFlux85 + 2*ReactionFlux87 - -2*ReactionFlux88 + ReactionFlux89 - ReactionFlux90 + ReactionFlux91)

d(Ecoli_4.luxI)/dt = 1/Ecoli_4*(ReactionFlux78 - ReactionFlux82)

d(Ecoli_4.luxR)/dt = 1/Ecoli_4*(ReactionFlux71 - ReactionFlux74 + ReactionFlux75 - ReactionFlux79)

d(Ecoli_4.AHL)/dt = 1/Ecoli_4*(2*ReactionFlux71 - -2*ReactionFlux74 + ReactionFlux77 - ReactionFlux81)

d(Ecoli_4.RFP)/dt = 1/Ecoli_4*(-ReactionFlux70 + ReactionFlux72)

d(Ecoli_4.lacI_mRNA)/dt = 1/Ecoli_4*(-ReactionFlux84 + ReactionFlux92)

d(Ecoli_4.luxI_mRNA)/dt = 1/Ecoli_4*(-ReactionFlux83 + ReactionFlux86 + ReactionFlux95)

d(Ecoli_4.luxR_mRNA)/dt = 1/Ecoli_4*(ReactionFlux76 - ReactionFlux80 + ReactionFlux96)

d(Ecoli_4.RFP_mRNA)/dt = 1/Ecoli_4*(ReactionFlux73 - ReactionFlux100)

d(Ecoli_4.lactose)/dt = 1/Ecoli_4*(ReactionFlux89 - ReactionFlux90)

d(Ecoli_4.[lacI-lactose])/dt = 1/Ecoli_4*(-ReactionFlux89 + ReactionFlux90)

d(Ecoli_4.Plac)/dt = 1/Ecoli_4*(ReactionFlux87 - ReactionFlux88)

d(Ecoli_4.[Plac-2lacI])/dt = 1/Ecoli_4*(-ReactionFlux87 + ReactionFlux88)

d(Ecoli_4.[luxR-2AHL])/dt = 1/Ecoli_4*(-ReactionFlux71 + ReactionFlux74)

b. fluxes:

ReactionFlux70 = (beta_8*Ecoli_4.RFP)*Ecoli_4

ReactionFlux71 = (K5off*Ecoli_4.[luxR-2AHL])*Ecoli_4

ReactionFlux72 = (Kt*Ecoli_4.RFP_mRNA)*Ecoli_4

ReactionFlux73 = (k8_4*N*kp)*Ecoli_4

ReactionFlux74 = (K5on*Ecoli_4.luxR*Ecoli_4.AHL*Ecoli_4.AHL)*Ecoli_4

ReactionFlux75 = (Kt*Ecoli_4.luxR_mRNA)*Ecoli_4
ReactionFlux76 = (k1*N)*Ecoli_4
ReactionFlux77 = (k15*Ecoli_4.luxI)*Ecoli_4
ReactionFlux78 = (Kt*Ecoli_4.luxI_mRNA)*Ecoli_4
ReactionFlux79 = (beta_7*Ecoli_4.luxR)*Ecoli_4
ReactionFlux80 = (beta_3*Ecoli_4.luxR_mRNA)*Ecoli_4
ReactionFlux81 = (beta_2*Ecoli_4.AHL)*Ecoli_4
ReactionFlux82 = (beta_6*Ecoli_4.luxI)*Ecoli_4
ReactionFlux83 = (beta_3*Ecoli_4.luxI_mRNA)*Ecoli_4
ReactionFlux84 = (beta_3*Ecoli_4.lacI_mRNA)*Ecoli_4
ReactionFlux85 = (beta_1*Ecoli_4.lacI)*Ecoli_4
ReactionFlux86 = (k7*(Ecoli_4.Plac/(Ecoli_4.Plac+Ecoli_4.[Plac-2lacI]))*N)*Ecoli_4
ReactionFlux87 = (K3off*Ecoli_4.[Plac-2lacI])*Ecoli_4
ReactionFlux88 = (K3on*Ecoli_4.lacI*Ecoli_4.lacI*Ecoli_4.Plac)*Ecoli_4
ReactionFlux89 = (K1off*Ecoli_4.[lacI-lactose])*Ecoli_4
ReactionFlux90 = (K1on*Ecoli_4.lacI*Ecoli_4.lactose)*Ecoli_4
ReactionFlux91 = (Kt*Ecoli_4.lacI_mRNA)*Ecoli_4
ReactionFlux92 = (k1*N)*Ecoli_4
ReactionFlux95 = (k8_4*kp*N)*Ecoli_4
ReactionFlux96 = (k8_4*kp*N)*Ecoli_4
ReactionFlux100 = (beta_3*Ecoli_4.RFP_mRNA)*Ecoli_4

For simplified equations:

$$\frac{dlacI}{dt} = -\beta_1 \times lacI + 2 \times K_{3off} \times [Plac - 2lacI] - 2 \times K_{3on} \times lacI \times Ecoli\_4.Plac + K_{1off} \times [lacI - lactose] - K_{1on} \times lacI \times lactose + K_t \times lacI\_mRNA$$

$$\frac{dluxI}{dt} = K_t \times luxI\_mRNA - \beta_6 \times luxI$$

$$\frac{dluxR}{dt} = K_{5off} \times [luxR - 2AHL] - K_{5on} \times luxR \times AHL^2 + K_t \times luxR\_mRNA - \beta_7 \times luxR$$

$$\frac{dAHL}{dt} = 2 \times K_{5off} \times [luR - 2AHL] - K_{5on} \times luxR \times AHL^2 + k_{15} \times luxI - \beta_2 \times AHL$$

$$\frac{dRFP}{dt} = -\beta_8 \times RFP + K_t \times RFP\_mRNA$$

$$\frac{dlacI\_mRNA}{dt} = -\beta_3 \times lacI\_mRNA + k_1 \times N$$

$$\frac{luxI\_mRNA}{dt} = -\beta_3 \times luxI\_mRNA + k_7 \times N \times \frac{Plac}{Plac+[Plac-2lacI]} + k_8\_4 \times k_p \times N$$

$$\frac{dluxR\_mRNA}{dt} = k_1 \times N - \beta_3 \times luxR\_mRNA + k_8\_4 \times k_p \times N$$

$$\frac{dRFP\_mRNA}{dt} = k_8\_4 \times N \times k_p - \beta_3 \times RFP\_mRNA$$

$$\frac{dlactose}{dt} = K_{1off} \times [lacI - lactose] - K_{1on} \times lacI \times lactose$$

$$\frac{d[lacI-lactose]}{dt} = -K_{1off} \times [lacI - lactose] + K_{1on} \times lacI \times lactose$$

$$\frac{dPlac}{dt} = K_{3off} \times [Plac - 2lacI] - K_{3on} \times lacI^2 \times Plac$$

$$\frac{d[Plac-2lacI]}{dt} = -K_{3off} \times [Plac - 2lacI] + K_{3on} \times lacI^2 \times Plac$$

$$\frac{d[luxR-2AHL]}{dt} = -K_{5off} \times [luxR - 2AHL] + K_{5on} \times luxR \times AHL^2$$

c. Initial coditions:

Ecoli_4.lacI = 6e-05 molarity

Ecoli_4.luxI = 0 molarity

Ecoli_4.luxR = 6e-05 molarity

Ecoli_4.AHL = 0 molarity

Ecoli_4.RFP = 0 molarity

Ecoli_4.lacI_mRNA = 1.6e-05 molarity

Ecoli_4.luxI_mRNA = 0 molarity

Ecoli_4.luxR_mRNA = 1.6e-05 molarity

Ecoli_4.RFP_mRNA = 0 molarity

Ecoli_4.lactose = 0.0023 molarity

Ecoli_4.[lacI-lactose] = 0 molarity

Ecoli_4.Plac = 0 molarity

Ecoli_4.[Plac-2lacI] = 4e-09 molarity

Ecoli_4.[luxR-2AHL] = 0 molarity

Ecoli_1.Pcons = 4e-09 molarity

Ecoli_2.Pcons = 4e-09 molarity

Ecoli_3.Pcons = 4e-09 molarity

Ecoli_4.Pcons = 4e-09 molarity

Ecoli_4.Prlux = 4e-09 molarity

k8_4 = 5e-06 molarity

d. when Ecoli_4.[luxR-2AHL] > Km, set k8_4 = k8

(5). Parameter Values(common used):

k1 = 1.25 1/second

beta_1 = 0.004 1/second

beta_2 = 0.00038 1/second

beta_3 = 0.000289 1/second

beta_6 = 0.000278 1/second

beta_7 = 0.000385 1/second

K1on = 11000 liter/(mole*second)

K1off = 0.011 1/second

K3on = 5e+10 (liter*liter)/(mole*mole*second)

K3off = 0.75 1/second

K5on = 5e+08 (liter*liter)/(mole*mole*second)

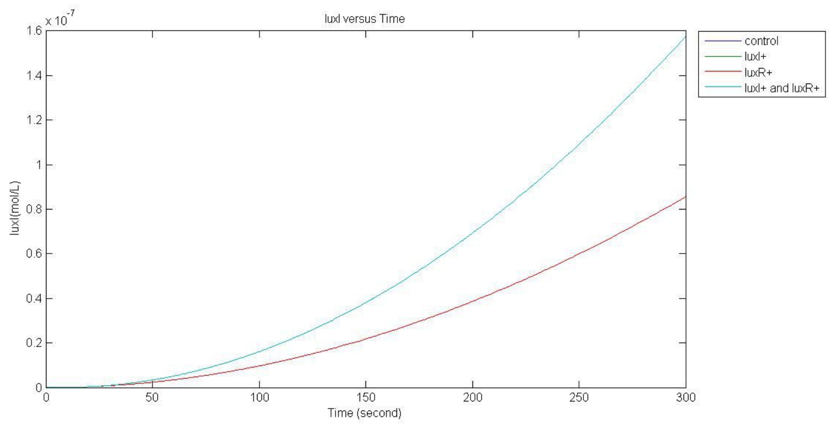
K5off = 5 1/second

Km = 1.3e-11 molarity
k8 = 0.28 molarity
Kt = 0.0017 1/second
k7 = 0.3 1/second
k15 = 0.06 1/second
N = 4e-09 molarity
beta_8 = 0.0001 1/second
C = 1e-22 molarity
kp = 1 liter/(mole*second)
E = -1e-22 molarity
Ecoli_1 = 2e-15 liter
Ecoli_2 = 2e-15 liter
Ecoli_3 = 2e-15 liter
Ecoli_4 = 2e-15 liter

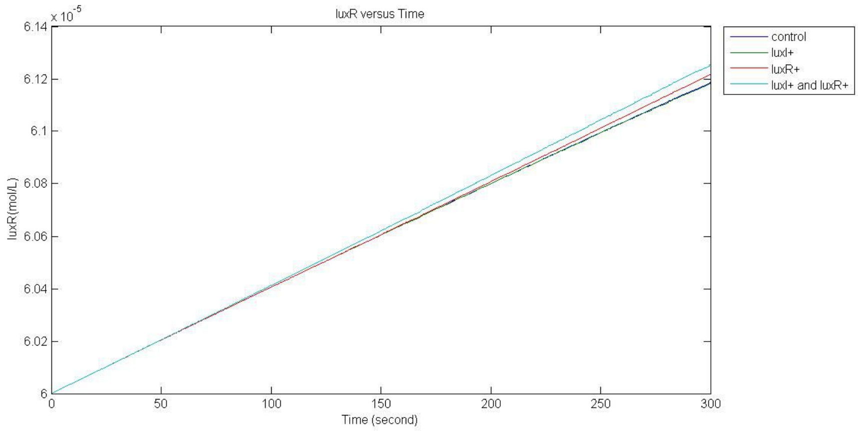
(6). Attention :

1. we make sure all values are positive.
2. we ignore the RFP_mRNA produced by Prlux without luxR-2AHL.

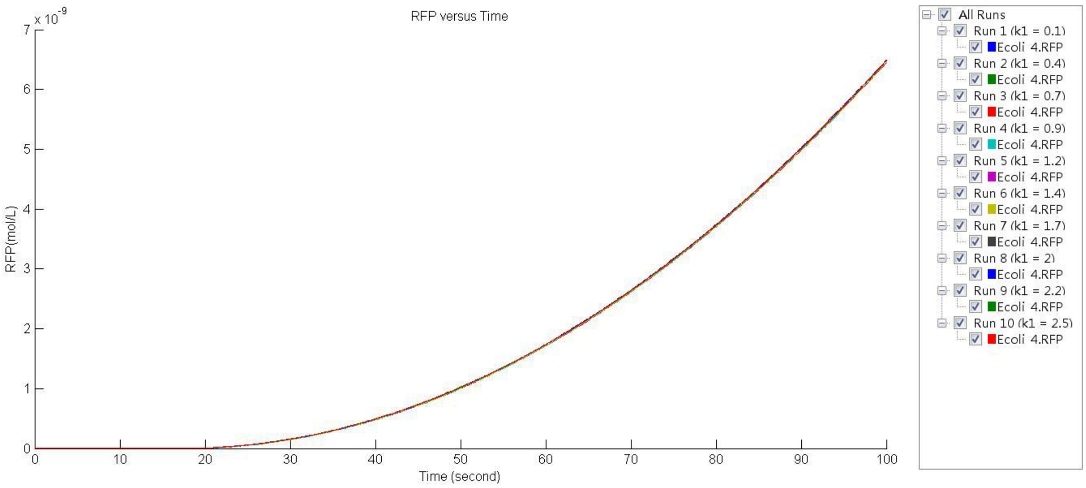
(7). Some more results



From this diagram, we can see the effect of feedback on luxI. It's clear that the concentration of luxI in binary system and luxI+ system is higher than that in luxR+ system and control system.

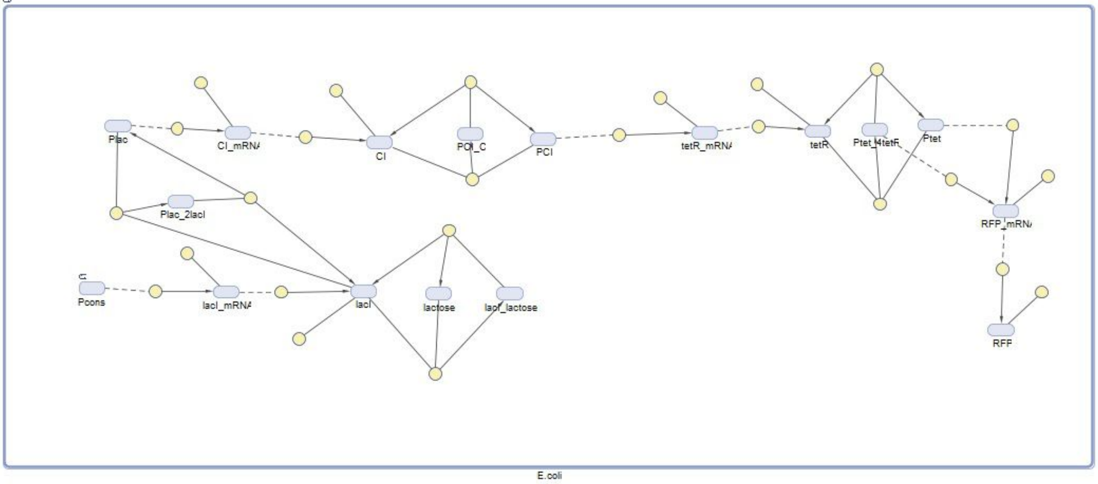


From this diagram, we can see the effect of feedback on luxR. The result is $RFP_{binary} > RFP_{luxR+} > RFP_{luxR+} = RFP_{control}$. By positive feedback, luxR+ system and binary system is better than the other two, but binary system has a stronger positive feedback than luxR+ system.



We aslo get some conclusion about the rubustness of the system. It is obvious that the strength of Pcons has a weak effect on the performance of the system, which means we don't need to struggle to find a so called best Pcons.

2. Time sensor



(1)The ODEs are listed as follows[5].

$$\frac{dPlac}{dt} = -K_{3on} \times Plac \times lacI^2 + K_{3off} \times Plac\_2lacI$$

$$\frac{dCI}{dt} = K_t \times CI\_mRNA - K_{6on} \times CI \times PCI - \beta_4 \times CI + K_{6off} \times PCI\_CI$$

$$\frac{dCI\_mRNA}{dt} = k_7 \times Plac - \beta_3 \times CI\_mRNA$$

$$\frac{dPCI}{dt} = -K_{6on} \times CI \times PCI + K_{6off} \times PCI\_CI$$

$$\frac{dtetR}{dt} = K_t \times tetR\_mRNA - \beta_5 \times tetR - 4 \times K_{7on} \times tetR^4 \times Ptet + 4 \times K_{7off} \times Ptet\_4tetR$$

$$\frac{dtetR\_mRNA}{dt} = k_{12} \times PCI - \beta_3 \times tetR\_mRNA$$

$$\frac{dPtet}{dt} = -K_{7on} \times tetR^4 \times Ptet + K_{7off} \times Ptet\_4tetR$$

$$\frac{dRFP}{dt} = K_t \times RFP\_mRNA - \beta_8 \times RFP$$

$$\frac{dRFP\_mRNA}{dt} = k_{13} \times Ptet - \beta_3 \times RFP\_mRNA + k_{14} \times Ptet\_4tetR$$

$$\frac{dlacI}{dt} = K_t \times lacI\_mRNA - \beta_1 \times lacI - 2 \times K_{3on} \times Plac \times lacI^2 + 2 \times K_{3off} \times Plac\_2lacI$$

$$-K_{1on} \times lacI \times lactose + K_{1off} \times lacI\_lactose$$

$$\frac{dlacI\_mRNA}{dt} = k_1 \times Pcons - \beta_3 \times lacI\_mRNA$$

$$\frac{dlactose}{dt} = -K_{1on} \times lacI \times lactose + K_{1off} \times lacI\_lactose$$

$$\frac{dlacI\_lactose}{dt} = K_{1on} \times lacI \times lactose - K_{1off} \times lacI\_lactose$$

---

[5] For more details about the ODES, please see the attachments.

$$\frac{dPlac\_2lacI}{dt} = K_{3on} \times Plac \times lacI^2 - K_{3off} \times Plac\_2lacI$$

$$\frac{dPCI\_CI}{dt} = K_{6on} \times CI \times PCI - K_{6off} \times PCI\_CI$$

$$\frac{dPtet\_4tetR}{dt} = K_{7on} \times tetR^4 \times Ptet - K_{7off} \times Ptet\_4tetR$$

(2)We also conclude some initial values by modeling[6].

Plac = 0 molarity
CI = 0 molarity
CI_mRNA = 0 molarity
PCI = 4e-09 molarity
tetR = 2.8e-05 molarity
tetR_mRNA = 4e-06 molarity
Ptet = 4e-09 molarity
RFP = 2.8e-06 molarity
RFP_mRNA = 3.7e-07 molarity
lacI = 6e-05 molarity
lacI_mRNA = 1.6e-05 molarity
lactose = 0.0023 molarity
lacI_lactose = 0 molarity
Plac_2lacI = 4e-09 molarity
PCI_CI = 0 molarity
Ptet_4tetR = 0 molarity
Pcons = 4e-09 molarity

(3) Parameter Values

k1 = 1.25 1/second
kT = 0.0017 1/second
beta3 = 0.000288 1/second
beta1 = 0.004 1/second
K1on = 11000 liter/(mole*second)
K3on = 5e+10 (liter*liter)/(mole*mole*second)
beta4 = 0.000935 1/second
K6on = 5e+10 liter/(mole*second)
k12 = 0.23 1/second
beta5 = 2.78e-05 1/second
k13 = 0.03 1/second
beta8 = 0.0001 1/second
K1off = 0.011 1/second
K3off = 0.75 1/second
K6off = 2000 1/second

6 Initial values here stand for concentrations of substances before adding lactose.

k7 = 0.3 1/second

K7on = 5e+10 (liter*liter*liter*liter)/(mole*mole*mole*mole*second)

K7off = 450 1/second

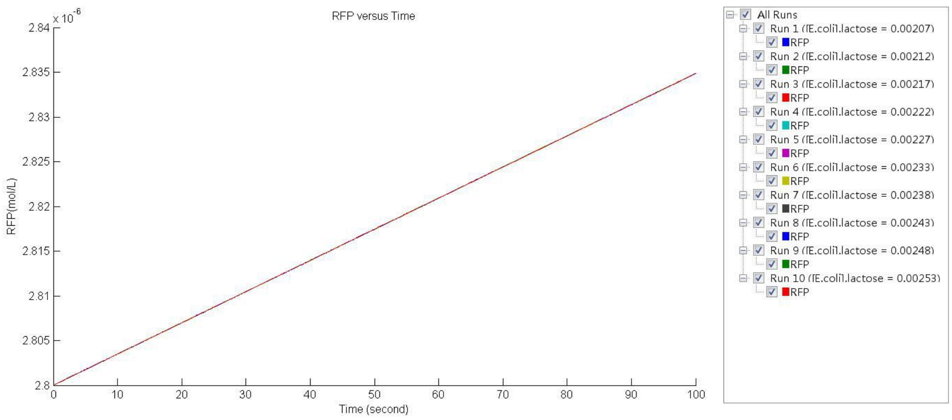
C = 1e-22 molarity

k14 = 2.1e-05 1/second

[E.coli] = 2e-15 liter

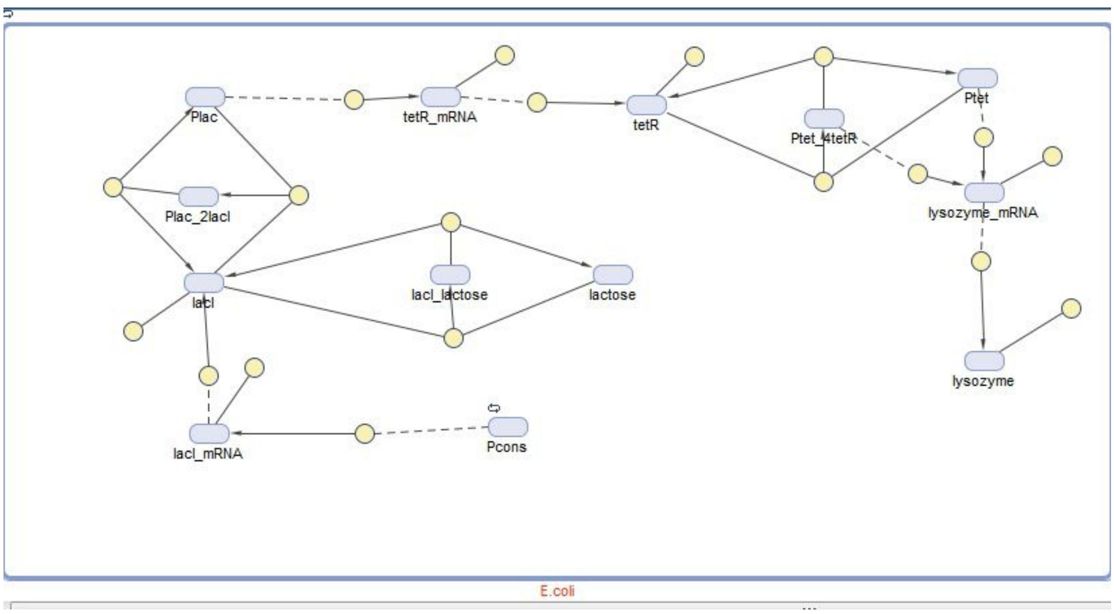
(4) We make sure all the values are positive.

(5) Some more results.



The robustness of the system is always important, especially the system of time sensor. From this diagram, we know that the RFP concentration can be hardly changed with the change of Pcons strength.

3. Suicide system.



(1) The ODEs are listed as follows.[7]

$$\frac{dPtet}{dt} = -K_{7on} \times tetR^4 \times Ptet + K_{7off} \times Ptet\_4tetR$$

$$\frac{dlysozyme\_mRNA}{dt} = k_{12} \times Ptet - \beta_3 \times lysozyme\_mRNA + k_{14} \times Ptet\_4tetR$$

$$\frac{dlysozyme}{dt} = K_t \times lysozyme\_mRNA - \beta_9 \times lysozyme$$

$$\frac{dPlac}{dt} = -K_{3on} \times lacI^2 \times Plac + K_{3off} \times Plac\_2lacI$$

$$\frac{dtetR\_mRNA}{dt} = k_7 \times Plac - \beta_3 \times tetR\_mRNA$$

$$\frac{dtetR}{dt} = K_t \times tetR\_mRNA - \beta_5 \times tetR - 4 \times K_{7on} \times tetR^4 \times Ptet + 4 \times K_{7off} \times Ptet\_4tetR$$

$$\frac{dlacI\_mRNA}{dt} = k_1 \times Pcons - \beta_3 \times lacI\_mRNA$$

$$\frac{dlacI}{dt} = K_t \times lacI\_mRNA - \beta_1 \times lacI - K_{1on} \times lacI \times lactose + K_{1off} \times lacI\_lactose$$
$$-2 \times K_{3on} \times lacI^2 \times Plac + 2 \times K_{3off} \times Plac\_2lacI$$

$$\frac{dlactose}{dt} = -K_{1on} \times lacI \times lactose + K_{1off} \times lacI\_lactose$$

$$\frac{dlacI\_lactose}{dt} = K_{1on} \times lacI \times lactose - K_{1off} \times lacI\_lactose$$

$$\frac{dPlac\_2lacI}{dt} = K_{3on} \times lacI^2 \times Plac\_2lacI$$

$$\frac{dPtet\_4tetR}{dt} = K_{7on} \times tetR^4 \times Ptet - K_{7off} \times Ptet\_4tetR$$

(2)We also conclude some initial values by modeling[8].

Ptet = 4e-09 molarity
lysozyme_mRNA = 1.8e-07 molarity
lysozyme = 5e-07 molarity
Plac = 4e-09 molarity
tetR_mRNA = 2.5e-06 molarity
tetR = 5.4e-06 molarity
lacI_mRNA = 1e-05 molarity
lacI = 1.35e-08 molarity
lactose = 0 molarity
lacI_lactose = 9e-05 molarity
Plac_2lacI = 0 molarity
Ptet_4tetR = 0 molarity

[7] For more details about the ODES, please refer to the attachment.
[8] Initial values here stand for concentration of substances before the removing lactose.

Pcons = 4e-09 molarity

(3) Parameter Values:

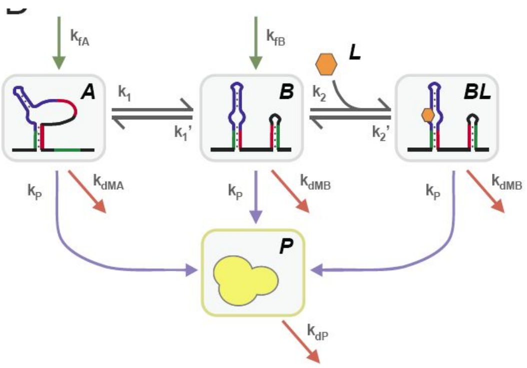
k1 = 1.25 1/second
beta3 = 0.000288 1/second
beta1 = 0.004 1/second
kT = 0.0017 1/second
K1on = 11000 liter/(mole*second)
K1off = 0.011 1/second
K3on = 5e+10 (liter*liter)/(mole*mole*second)
K3off = 0.75 1/second
k7 = 0.3 1/second
beta5 = 0.000278 1/second
K7on = 5e+10 (liter*liter*liter*liter)/(mole*mole*mole*mole*second)
K7off = 450 1/second
k12 = 0.023 1/second
beta9 = 0.0001 1/second
C = 1e-22 molarity
N = 4e-09 molarity
k14 = 2.1e-05 1/second
B = 9e-05 molarity
[E.coli] = 2e-15 liter

(4) Make sure all the values positive

4. Riboswitch evaluation

(1) some prepares

The kinetics diagram1 of riboswitch[9]



The kinetics diagram2 of riboswitch[10]

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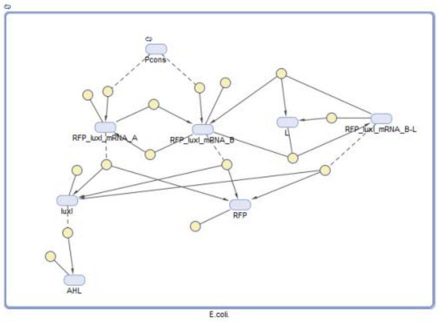
[9] From reference [28]
[10] The diagram can be made by Simbiology from a constructed model.



The ranges of some parameters used in our model[11]

Parameter	Units	Value Range	References
k_f	M/s	$10^{-13} – 10^{-8}$	(Voigt *et al*, 2005)
k_P	1/s	$10^{-4} – 10^{1}$	(Voigt *et al*, 2005)
$k_E{}^{\alpha}$	1/s	$10^{-2} – 10^{2}$	(Pan and Sosnick, 2006; Wickiser *et al*, 2005b)
k_1, k_1'	1/s	$10^{-3} – 10^{3}$	(Lee *et al*, 2007; Su *et al*, 2005; Zarrinkar *et al*, 1996; Zhuang *et al*, 2000)
k_2	1/M·s	$10^{3} – 10^{8}$	(Greenleaf *et al*, 2008; Kensch *et al*, 2000; Lang *et al*, 2007; Rieder *et al*, 2007; Wickiser *et al*, 2005a; Wickiser *et al*, 2005b; Win *et al*, 2006)
k_2'	1/s	$10^{-3} – 10^{1}$	(Greenleaf *et al*, 2008; Kensch *et al*, 2000; Wickiser *et al*, 2005a; Wickiser *et al*, 2005b; Win *et al*, 2006)
k_M	1/s	$10^{-2} – 10^{-1}$	(Crothers *et al*, 1974; Wickiser *et al*, 2005b)
k_{dM} (norm)$^{\beta}$	1/s	$10^{-5} – 10^{-2}$	(Bernstein *et al*, 2002; Leclerc *et al*, 2002; Narsai *et al*, 2007; Selinger *et al*, 2003)
k_{dM} (rib)$^{\gamma}$	1/s	10^{-1}	(Emilsson *et al*, 2003)
$k_{dP}{}^{\beta}$	1/s	$10^{-5} – 10^{-2}$	(Belle *et al*, 2006; Corish and Tyler-Smith, 1999)

The table of parameters used in riboswitch model(MC10)

Name	Description	Value	Ref
kfA	Half of The transcription rates of Pcons1	0.625/s	estimation [12] and [10]
kfB	Half of The transcription rates of Pcons1	0.625/s	estimation [13] and [10]
k1	The change rates from A to B	2.44/s	Estimation

[11] From reference [28]

[12] Actually, the transcription rates of Anderson family promoter is measured by the intensity of relative fluorescence in TG1 grown in LB media to saturation. BBa_J23119 is the only one without measurement. However， it can be estimated from some paper.

[13] Actually, the transcription rates of Anderson family promoter is measured by the intensity of relative fluorescence in TG1 grown in LB media to saturation. BBa_J23119 is the only one without measurement. However， it can be estimated from some paper.

k1’	The change rates from B to A	0.409/s	Estimation
k2	Binding rates of the ligand	10^5	Estimation
k2’	Dissociation rates of the lipid and mRNA complex	0.57	Estimation
kdMA	The degradation rates of mRNA A	0.0167/s	[26]
kdMB	The degradation rate of mRNA B	0.288*10^-3/s	[6]
kp	The translating rates of mRNA	0.0017/s	[19]
Kdp	The degradation rates of RFP	1.0*10^-4/s	Estimation
N	The concentration of gene	4*10^-9 mol/L	Estimation

The table or K1 and Kd

Number	E（combine,B）kcal/mol	E （ not,A ） kcal/mol	K1=k1/k1’	Kd=k2’/k2(umol/L)
MC10	-24.7	-23.6	5.96	5.7*
MC1	-39.2	-31	6.04*10^5	8.4
MC26	-35.7	-32	406	4.6*
MC31	-41.8	-37.1	2059	4.6*
MC7	-24.6	-19.7	2848	3.0**
MC3	-36.9	-30.8	19981	3.0**
MC14	-26.9	-21.6	5452	5.7*

The table to get k1, k1’, k2, k2’

Number	K1=k1/k1’	k1***(1/s)	k1’***(1/s)	Kd=k2’/k2(umol/L)	k2***(L/mol*s)	k2’***(1/s)
MC10	5.96	2.44	0.409	5.7*	10^5	0.57
MC1	6.04*10^5	777	1.29*10^-3	8.4	10^5	0.84
MC26	406	20.1	0.0495	4.6*	10^5	0.46
MC31	2059	45.4	0.0220	4.6*	10^5	0.46
MC7	2848	53.4	0.0188	3.0**	10^5	0.3
MC3	19981	141	0.00706	3.0**	10^5	0.3
MC14	5452	73.8	0.0135	5.7*	10^5	0.57

Attention:

*:estimated by the secondary structure.([24])

**:almost assumption.

***:assumption

(2) The content of formula and results

The table 1 of formulas for getting evaluating values[14]

Performance descriptor	General equation
η	$\frac{k_f}{k_{dP}} \cdot \frac{K_1\gamma_1}{K_1\gamma_1 + \frac{k_{dMB}}{k_{dMA}}} \left(\frac{k_{fA}\gamma_1 + k_{fB}}{k_f}\right) \left\lvert \frac{K_A}{k_{dMA}} - \frac{K_B}{k_{dMB}} \right\rvert$
EC_{50}	$\frac{1 + K_1\gamma_1 \frac{k_{dMA}}{k_{dMB}}}{K_2\gamma_2}$
P(L=0)	$\frac{k_f}{k_{dP}} \cdot \left[\frac{k_{fA}}{k_f}\frac{K_A}{k_1}\gamma_1 + \frac{K_A K_1\gamma_1 + K_B}{k_{dMA}K_1\gamma_1 + k_{dMB}}\left(\frac{k_{fA}\gamma_1 + k_{fB}}{k_f}\right)\right]$
P(L→∞)	$\frac{k_f}{k_{dP}} \cdot \left[\frac{k_{fA}}{k_f}\frac{K_A}{k_1}\gamma_1 + \frac{K_B}{k_{dMB}}\left(\frac{k_{fA}\gamma_1 + k_{fB}}{k_f}\right)\right]$

The table 2 of formulas for getting evaluating values[15]

14 From reference [28]

15 From reference [28]

Model parameter	Regulatory Mechanism TR	TT	MD
k_{iA}	k_{dM}	k_M	k_{dMA}
k_{iB}			k_{dMB}
K_A	k_{PA}	$k_P \frac{k_{MA}}{k_M}$	k_P
K_B	k_{PB}	$k_P \frac{k_{MB}}{k_M}$	
γ_1	$\frac{k_1}{k_1 + k_{dM}}$	$\frac{k_1}{k_1 + k_M}$	$\frac{k_1}{k_1 + k_{dMA}}$
γ_2	$\frac{k_2'}{k_2' + k_{dM}}$	$\frac{k_2'}{k_2' + k_M}$	$\frac{k_2'}{k_2' + k_{dMB}}$

The result of evaluating values of riboswitches by formula

number	η	EC50	P(L=0)	P(L->∞)
MC10	2.620E-04	8.972E-05	3.217E-05	2.942E-04
MC1	2.784E-08	5.703E-06	2.951E-04	2.951E-04
MC26	3.621E-05	5.260E-06	2.588E-04	2.950E-04
MC31	7.940E-06	4.732E-06	2.871E-04	2.951E-04
MC7	5.785E-06	3.064E-06	2.893E-04	2.951E-04
MC3	8.392E-07	3.012E-06	2.943E-04	2.951E-04
MC14	3.051E-06	5.764E-06	2.921E-04	2.951E-04

(3) The content of model.

a. ODEs:

d(RFP_luxI_mRNA_A)/dt = 1/E.coli.*(ReactionFlux1 - ReactionFlux5 - ReactionFlux8 + ReactionFlux9)

d(RFP_luxI_mRNA_B)/dt = 1/E.coli.*(ReactionFlux2 - ReactionFlux3 + ReactionFlux4 - ReactionFlux6 + ReactionFlux8 - ReactionFlux9)

d([RFP_luxI_mRNA_B-L])/dt = 1/E.coli.*(ReactionFlux3 - ReactionFlux4 - ReactionFlux7)

d(L)/dt = 1/E.coli.*(-ReactionFlux3 + ReactionFlux4 + ReactionFlux7)

d(RFP)/dt = 1/E.coli.*(ReactionFlux10 + ReactionFlux11 + ReactionFlux12 - ReactionFlux14)

d(luxI)/dt = 1/E.coli.*(ReactionFlux10 + ReactionFlux11 + ReactionFlux12 - ReactionFlux13)

d(AHL)/dt = 1/E.coli.*(ReactionFlux15 - ReactionFlux16)

Fluxes:

ReactionFlux1 = (kfA*Pcons)*[E.coli.]

ReactionFlux2 = (kfB*Pcons)*[E.coli.]

ReactionFlux3 = (k2*RFP_luxI_mRNA_B*L)*[E.coli.]

ReactionFlux4 = ([k2']*[RFP_luxI_mRNA_B-L])*[E.coli.]

ReactionFlux5 = (kdMA*RFP_luxI_mRNA_A)*[E.coli.]

ReactionFlux6 = (kdMB*RFP_luxI_mRNA_B)*[E.coli.]

ReactionFlux7 = (kdMB*[RFP_luxI_mRNA_B-L])*[E.coli.]

ReactionFlux8 = (k1*RFP_luxI_mRNA_A)*[E.coli.]

ReactionFlux9 = ([k1']*RFP_luxI_mRNA_B)*[E.coli.]

ReactionFlux10 = (kp*RFP_luxI_mRNA_A)*[E.coli.]

ReactionFlux11 = (kp*RFP_luxI_mRNA_B)*[E.coli.]

ReactionFlux12 = (kp*[RFP_luxI_mRNA_B-L])*[E.coli.]

ReactionFlux13 = (beta6*luxI)*[E.coli.]

ReactionFlux14 = (kdp*RFP)*[E.coli.]

ReactionFlux15 = (k15*luxI)*[E.coli.]

ReactionFlux16 = (beta2*AHL)*[E.coli.]

b. Parameter Values:

kfA = 0.625 1/second

kfB = 0.625 1/second

k1 = 45.4 1/second

[k1'] = 0.022 1/second

k2 = 1e+05 liter/(mole*second)

[k2'] = 0.46 1/second

kdMA = 0.0167 1/second

kdMB = 0.000288 1/second

kp = 0.0017 1/second

kdp = 0.0001 1/second

N = 4e-09 molarity

beta2 = 0.00038 1/second

beta6 = 0.000278 1/second

k15 = 0.06 1/second

[E.coli.] = 2e-15 liter

c. Initial Conditions:

RFP_luxI_mRNA_A = 0 molarity

RFP_luxI_mRNA_B = 0 molarity

[RFP_luxI_mRNA_B-L] = 0 molarity

L = 0.01 molarity

RFP = 0 molarity

luxI = 0 molarity

AHL = 0 molarity

Pcons = 4e-09 molarity

5. Program of riboswitch and positive feedback was listed in the supplement. However, because of limit of space, we didn’t list the program of suicide and time sensor.